

AMENDMENTS TO THE CLAIMS

Please amend the claims as follows:

Claim 1 (Currently Amended): A method for detecting, separating and identifying an expressed trace protein and/or peptide in a test sample, comprising:

converting a protein and/or peptide in a test sample to a fluorescent derivative by labeling said protein and/or peptide with a fluorescent derivation reagent, wherein said fluorescent derivation reagent is non-fluorescent itself,

subjecting the labeled protein and/or peptide to one-dimensional or two-dimensional HPLC/fluorescence detection, separating said fluorescent derivative by a combination of HPLC and fluorescence detection (HPLC/fluorescence detection) to obtain fluorescent fractions,

applying the fluorescent fractions ~~to mass spectrometry or MS/MS analysis, or applying the fluorescent fractions~~ to enzymatic hydrolysis to obtain digested peptide fragments,

~~separating the peptide fragments to obtain peptide fractions,~~

subjecting the digested peptide fragments to second stage HPLC/fluorescence detection to obtain a fluorescent chromatogram,

applying ~~[[the]]~~ peptide fractions of the fluorescent chromatogram to mass spectrometry or MS/MS analysis,

collating the mass spectrometry or MS/MS data with a database, and

providing said collated data for structural analysis to identify the expressed protein and/or peptide.

Claim 2 – 3 (Canceled):

Claim 4 (Previously Presented): The method according to claim 1, wherein a functional group-specific fluorescence reagent is added to an aqueous solution of the protein and/or peptide sample, and a surfactant and/or protein denaturing agent is optionally added, to fluorescently label the protein and/or peptide.

Claim 5 (Currently Amended): The method according to claim 1, wherein the fluorescent derivative is applied to a separation means of a HPLC/fluorescence detection selected from the group consisting of an ion exchange column HPLC equipped with a fluorescence detector, a reverse phase partition HPLC equipped with a fluorescence detector, a gel filtration HPLC equipped with a fluorescence detector, ~~and electrophoresis~~, and a peak fraction thereof is captured while monitoring fluorescence.

Claim 6 (Currently Amended): The method according to claim 1, wherein the fluorescent fraction is ~~subjected~~ applied to enzymatic hydrolysis using a protease selected from the group consisting of a peptidase, a trypsin and a chymotrypsin.

Claim 7 (Currently Amended): The method according to claim 1, wherein the ~~enzymatic hydrolysis product is~~ digested peptide fragments are applied to reverse phase HPLC equipped with a fluorescence detector to detect a fluorescence peak, and mass spectrometry or MS/MS analysis is carried out on fluorescence-labeled fragments and non-fluorescence-labeled fragments.

Claim 8 (Canceled).

Claim 9 (Previously Presented): The method according to claim 1, wherein the test sample is a protein and/or peptide sample collected from a biological sample.

Claim 10 (Canceled):

Claim 11 (Currently Amended): A system for detecting, separating and identifying an expressed trace protein and/or peptide ~~used in the method~~ according to the method of claim 1, comprising

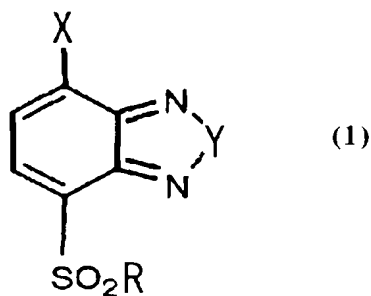
a first reactor for labeling a protein and/or peptide of a test sample with a ~~fluorescence~~ fluorescent derivation reagent, wherein said fluorescent derivation reagent is non-fluorescent itself,

a one-dimensional or two-dimensional HPLC equipped with a fluorescence detector for fluorescent fractionation of a fluorescent derivative labeled with the ~~fluorescence~~ fluorescent derivatization reagent,
a second reactor for enzymatic hydrolysis of the fluorescent fraction,
a second-stage HPLC equipped with a fluorescence detector for fluorescent detection of fluorescence-labeled fragments of the enzymatic hydrolysis product,
mass spectrometer or MS/MS analyzer, and
one or two or more types of structural analysis devices equipped with a database containing data on amino acids labeled with the fluorescence reagent.

Claim 12 (Original): The system according to claim 11, wherein the first reactor, the one-dimensional or two-dimensional HPLC equipped with a fluorescence detector, the second reactor, and the second-stage HPLC equipped with a fluorescence detector are arranged in series.

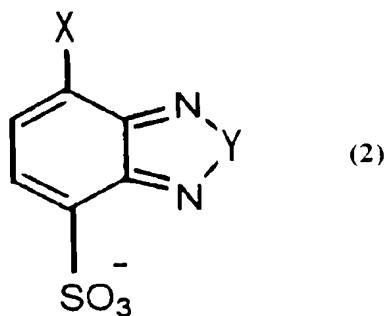
Claim 13 (Previously Presented): The method according to claim 1, wherein a protein and/or peptide in a test sample is converted to a fluorescent derivative by using as a fluorescent derivatization reagent

a compound represented by formula (1):



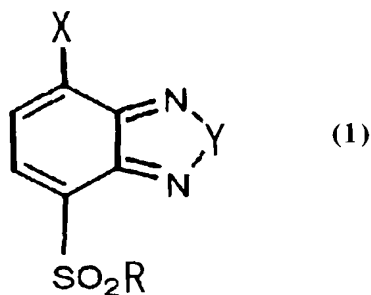
wherein X represents a halogen atom, Y represents O, Se or S, and R represents -NH₂, -NHR' (wherein, R' represents an alkyl-substituted N-alkyl group, dialkyl-substituted N-alkyl group or trialkyl-substituted N-alkyl group) or -NR''R''' (wherein, R'' represents an alkyl group, and R''' represents an alkyl-substituted N-alkyl group, dialkyl-substituted N-alkyl group or trialkyl-substituted N-alkyl group) or an isotope compound thereof, or

a compound represented by formula (2):

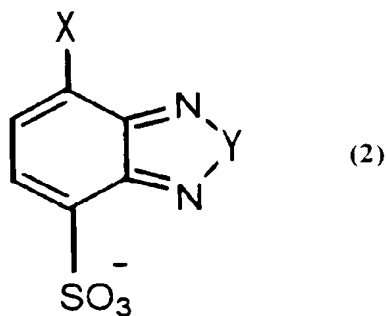


wherein X represents a halogen atom and Y represents Se or S, or an isotope compound thereof.

Claim 14 (Previously Presented): The method according to claim 1, wherein a protein and/or peptide in the method according to claim 1 is converted to a fluorescent derivative by using a fluorescent derivatization reagent used for fluorescent derivatization which is a compound represented by formula (1):



wherein X represents a halogen atom, Y represents O, Se or S, and R represents -NH₂, -NHR' (wherein, R' represents an alkyl-substituted N-alkyl group, dialkyl-substituted N-alkyl group or trialkyl-substituted N-alkyl group) or -NR''R''' (wherein, R'' represents an alkyl group, and R''' represents an alkyl-substituted N-alkyl group, dialkyl-substituted N-alkyl group or trialkyl-substituted N-alkyl group)[[]] or an isotope compound thereof, or a compound represented by formula (2):



wherein X represents a halogen atom and Y represents Se or S, or an isotope compound thereof.

Claim 15 (Canceled):

Claim 16 (Currently Amended): A method for detecting, separating and identifying a protein and/or peptide, ~~wherein~~ comprising

converting a protein and/or peptide in different test samples in the form of sample A and sample B ~~is converted~~ to a fluorescent derivative, respectively, with at least two fluorescent derivatization reagents, wherein said fluorescent derivation reagents are non-fluorescent themselves and have ~~having~~ different fluorescence wavelengths,

separating and detecting the fluorescent derivative ~~is separated and detected~~ with an HPLC equipped with a fluorescence detector, ~~and identification is carried out by~~

~~applying to quantification of each fluorescence peak either directly or collectively following fractionation and/or applying each fluorescence peak collectively to enzymatic hydrolysis, and followed by quantification of the hydrolysis product, or~~

applying the hydrolysis product to HPLC-mass spectrometry,

wherein the protein and/or peptide is converted to a derivative with at least two fluorescent derivatization reagents selected from the group consisting of DAABD-X, DAABSeD-X, DAABThD-X, an isotope of DAABD-X, an isotope of DAABSeD-X, and an isotope of DAABThD-X, wherein X represents Cl or F.

Claim 17 (Original): The method according to claim 16, wherein each fluorescence peak is applied to quantification by HPLC either directly or collectively, and the ratio of each derivative of the protein and/or peptide in sample A and sample B is calculated.

Claim 18 (Original): The method according to claim 16, wherein the hydrolysis product is applied to quantification by HPLC, and the ratio of each derivative of the protein and/or peptide in sample A and sample B is calculated.

Claim 19 (Original): The method according to claim 16, wherein the reaction product of a first fluorescent derivatization reagent and the reaction product of a second fluorescent derivatization reagent with the protein and/or peptide in sample A and sample B are combined, applied to two HPLC capable of excitation and fluorescence detection, applied to enzymatic hydrolysis after fractionating and combining each fluorescence peak, and identification is carried out by applying the hydrolysis product to HPLC-mass spectrometry.

Claim 20 (Original): The method according to claim 16, wherein samples A and B are two types of cell, tissue or body fluid samples.

Claims 21 – 26 (Canceled):